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39.

Guidelines for pre-transfusion compatibility procedures in blood transfusion laboratories

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1. INTRODUCTION

1.1. Purpose of pre-transfusion guidelines. Technical errors and/or inappropriate test systems or administrative errors may result in immediate and delayed haemolytic transfusion reactions. The purpose of these guidelines, which replace those previously published (BCSH, 1991a), is to define organizational, documentation and technical procedures undertaken in hospital or Regional Transfusion Centre laboratories prior to blood transfusion.

1.2. Elements in pre-transfusion testing

1.2.1. ABO and RhD grouping of the recipient

1.2.2. Antibody screen of the recipient, or mother in the case of neonatal transfusion, which in the event of a positive screen, should be followed by antibody identification.

1.2.3. A computer or manual check of records. These three elements constitute a group and screen.

1.2.4. Donor red cell selection and crossmatching.

1.2.5. In certain emergencies, the recipient's need for immediate red cell support may dictate that pre-transfusion testing is abbreviated.

1.3. Clinical significance of red cell antibodies

1.3.1. Clinically significant antibodies are those which are capable of giving rise to accelerated destruction of red cells bearing the relevant antigen.

1.3.2. Anti-A, anti-B and anti-A,B must always be regarded to be of clinical significance.

1.3.3. With few exceptions, irregular antibodies which are potentially clinically significant are only those which are reactive in the indirect antiglobulin test (IAT), performed strictly at 37°C.

1.3.4. In certain clinical emergencies, e.g. massive blood loss, the recipients need for red cell transfusion may necessitate the use of incompatible units.

1.3.5. The clinical significance of antibodies of given specificities and selection/testing of red cell units prior to their issue:

Specificity	Clinical significance	Selection of units
Rh antibodies (reactive in IAT)	Yes	Antigen negative
Kell antibodies	Yes	Antigen negative
Duffy antibodies	Yes	Antigen negative
Kidd antibodies	Yes	Antigen negative
Anti-S,-s	Yes	Antigen negative
Anti-A_1, -P_1, -N	Rarely	IAT crossmatch compatible 37°C
Anti-M,	Rarely	IAT crossmatch compatible 37°C
Anti-M reactive at 37°C	Sometimes	Antigen negative
Anti-Le^a, Anti-Le^{a+b}	Rarely	IAT crossmatch compatible 37°C
Anti-Le^b	No	Not clinically significant and can be ignored
High-titre low-avidity antibodies (HTLA)	Unlikely	Seek advice from Transfusion Centre
Antibodies against low/high-frequency antigens	Depends on specificity	Seek advice from Transfusion Centre

2. QUALITY ASSURANCE IN PRE-TRANSFUSION PROCEDURES

2.1. Role of the Hospital Transfusion Committee. This document is primarily concerned with the laboratory aspect of pre-transfusion testing. However, the provision of safe and effective red cell transfusion support requires multidisciplinary collaboration. The Hospital Transfusion Committee can help with the areas covered by this document, as follows. (i) Training medical staff and designated phlebotomists in accordance with local written procedures for the generation of request forms and labelling of patient samples. (ii) Supporting the Consultant Haematologist and Transfusion laboratory in enforcing policies relating to the non-laboratory aspects of blood transfusion, e.g. documentation, identification of patients and labelling of blood samples for transfusion, required intervals between samples, verbal requests, the collection of blood from the blood issue refrigerator and conditions of storage outside the transfusion laboratory. (iii) In the formulation and periodic review of Maximum Surgical Blood Order Schedules (MSBOS). These Schedules are essential if donor blood and laboratory staff are to be utilized effectively. (iv) Audit of practice.

2.2. Laboratory aspects of quality assurance

2.2.1. The laboratory should document its Quality System and include the following points.

2.2.1.1. The laboratory should participate in appropriate External Quality Assurance.

2.2.1.2. Transfusion laboratories should make use of systems validated against the documented requirements of the laboratory. They should also have written standards for the manual procedures which need to be followed when the computer system is unavailable.

2.2.1.3. Whether using a manual or semi-automated system, the laboratory must develop procedures to build in checks for all critical points in transfusion testing, e.g. preserving the identity of samples during separation and processing.

2.2.2. *Reagents.* (i) The head of the laboratory should refer to the specifications for reagents given in the Guidelines for the Blood Transfusion Services in the UK (Department of Health, 1994). (ii) All reagents or systems should be used in accordance with the manufacturer's instructions. If this is not appropriate, then the procedure should be validated in accordance with the BCSH Guidelines on evaluation, validation and implementation of new techniques for blood grouping, antibody screening and crossmatching (BCSH, 1995). (iii) There should be a record of all batch numbers and expiry dates of all reagents used in the laboratory.

2.2.3. *Techniques.* (i) All procedures used should be in accordance with recommended practice as outlined in Section 8. (ii) It is imperative that the antiglobulin technique chosen has been validated against the documented requirements of the laboratory and has been subjected to a thorough field trial before being introduced into the laboratory (Voak, 1992). (iii) All changes in techniques must also be thoroughly validated in accordance with the BCSH Guidelines on evaluation, validation and implementation of new techniques before being introduced into routine use (BCSH, 1995). (iv) Written authorised Standard Operating Procedures (SOPs) which cover all aspects of the laboratory work must be available and reviewed regularly. (v) The regular checking and maintenance of all laboratory equipment must be documented. In particular, there should be a documented Quality Assurance procedure for cell washers, e.g. using the NIBSC anti-D standard (Phillips *et al.*, 1993).

2.2.4. *Staff training and proficiency.* (i) There must be a documented programme for training laboratory staff which covers all SOPs in use and which fulfils the documented requirements of the laboratory. (ii) Laboratory tasks should only be undertaken by appropriately trained staff. (iii) There must be a documented programme for assessing staff proficiency, e.g. replicate testing for the IAT, which should include details of the action limits for retraining (Voak *et al.*, 1988).

2.2.5. *Auditing and reviewing practice.* (i) There should be a system in place for documenting and reviewing all incidents of noncompliance with procedures. All serious incidents of noncompliance, near misses and adverse transfusion reactions should be reviewed by the Hospital Transfusion Committee. There should be a mechanism for reporting the adverse effects of transfusion to the Consultant Haematologist. (ii) The systems should enable a full audit trail of laboratory steps, including the original results, interpretations, authorizations, and all staff responsible for conducting each step. (iii) A programme of independent audits should be conducted to assess compliance with documented 'in-house' laboratory procedures.

3. SAMPLES/DOCUMENTATION

3.1. Introduction. The majority of ABO-incompatible transfusions are due to clerical/documentation/identification errors (Sazama, 1990).

3.2. Written/electronic requests

3.2.1. Transfusions must be prescribed by a medical officer.

3.2.2. It is essential that the request form and sample

contain the following minimum patient identification (PIN) as described in BCSH Guidelines on Hospital Blood Bank Documentation and Procedures (BCSH, 1991b): (i) surname (correctly spelt); (ii) first name(s); (iii) date of birth (not age or year of birth); (iv) hospital number/accident and emergency number.

The sample should be labelled and signed by the person taking it.

A local SOP should be in place for the procedure for dealing with inadequately labelled samples.

3.2.3. Information concerning the sex of the patient and obstetric and recent transfusion history should be obtained wherever possible and is essential when there are anomalous pretransfusion testing results.

3.2.4. Requests should also include the date and time required, the number or volume and type of components required, the reason for request and any other specific requirements relating to the patient or request.

3.2.5. Addressograph labels are more likely to result in inadequate checking of patient identification at the bedside and it is therefore recommended that these are not accepted for grouping or pretransfusion testing samples.

3.2.6. Electronic ward requesting should comply to all the same minimum standards as in 3.2.1.–3.2.5.

3.2.7. Samples received from trauma or unconscious accident and emergency patients are unlikely to contain the full PIN. There must, however, be at least one unique identifier, usually an accident and emergency or trauma number and the sex of the patient. The sample should be taken and labelled and the form and sample signed by the prescribing medical officer as one continuous procedure.

In the event of there not being at least one unique identifier on the sample in a life-threatening situation, group O blood only must be issued until a suitably labelled sample is available. If the patient is a premenopausal female, group O RhD-negative blood should be given.

3.3. Telephone requests

3.3.1. There should be a policy for documenting telephone requests. The use of a telephone request pad is recommended.

3.3.2. Requests must be made by a medical officer or delegated individual. The identity of the person or people initiating and making the request should be documented.

3.3.3. The following minimum information must be given and confirmed: (i) surname; (ii) forename; (iii) hospital/accident and emergency number/trauma number; (iv) location; (v) number/volume and type of product; (vi) reason for request; (vii) date and time required.

3.4. Duplicate records. Duplicate patient records must be avoided otherwise essential transfusion or antibody history may be overlooked. It is therefore necessary, at the time of the request, to identify and link separate records that exist for each patient.

If a computer system is in use the user must be alerted at the entry of a request that there are existing records for patients with the same name and date of birth. If a computer system is not in use, manual records need to be checked by name and date of birth for previous encounters.

3.5. Sample requirements. 3.5.1. Clotted or EDTA samples may be used for pretransfusion testing. If a change in protocol is made from the use of serum to plasma, appropriate validation using weak examples of antibodies must be performed to ensure that the detection of clinically significant antibodies is not compromised.

3.6. Timing of sample collection in relation to previous transfusions

3.6.1. Transfusion or pregnancy may cause either a primary or secondary immune response and samples selected for crossmatching or antibody screening must take account of this, so that any newly developed antibodies are detected. It is also important to note that any component containing residual red cells can elicit an immune response.

In situations in which patients are being repeatedly transfused it is not necessary to require a daily sample. These patients should be screened for the development of irregular antibodies at least every 72 h.

If a transfusion has been given more than 72 h previously a new sample is required according to the following guidance:

Patient transfused within	Sample to be taken
3 to 14 days	24 h before transfusion
14 to 28 days	72 h before transfusion
28 days to 3 months	1 w before transfusion

It is recognized that for some individuals, e.g. thalassaemic patients who are repeatedly transfused and who have not had an antibody response, a more tolerant approach may be taken.

3.6.2. If there is no history of pregnancy or transfusion during the previous 6 months, stored plasma or serum may be used for crossmatching. Storage conditions will determine the length of storage. See section 3.7.

3.6.3. It is recognized that there may be a problem obtaining samples from pregnant women, who, for example, are booked for elective caesarian section, but may not arrive in the hospital until shortly before surgery. As immunization is more likely to occur

during the last trimester of pregnancy, samples used for pretransfusion testing should never be more than 7 days old. Where possible, it is advisable that a sample taken immediately before transfusion is also available for retrospective testing in the event of a transfusion reaction occurring.

3.7. Storage of samples. **3.7.1** Whole blood samples will deteriorate over a period of time. Problems associated with storage include red cell lysis, loss of complement in serum and decrease in potency of red cell antibodies, particularly IgM antibodies and bacterial contamination.

There is a paucity of evidence concerning the use of stored samples for pretransfusion testing. Laboratories may wish to evaluate the stability of weak antibodies before making their local recommendations for storage conditions. The following, however, are suggested as working limits:

	18–25°C	4°C	−30°C
EDTA whole blood	Up to 48 h	Up to 7 days	N/A
Separated plasma/ serum	N/A	Up to 7 days	6 months

4. ABO AND RHD GROUPING

4.1 Introduction

4.1.1. Samples must be grouped for ABO and RhD using a validated technique. See also section 3.6.

4.1.2. Testing of patient's red cells against blood grouping reagents *and* of patient's plasma/serum against known reagent red cells (reverse group) should be performed, wherever possible, to determine the ABO group of all patients over 6 months of age.

4.2. ABO grouping

4.2.1. Patient's red cells should be tested against monoclonal anti-A and anti-B blood grouping reagents. See section 4.8.1.

4.2.2. Patient's plasma or serum should be tested against A_1 and B reagent red cells. The reverse group should include a negative control, e.g. patients own cells or group O cells, to exclude reactions with A and B cells due to cold antibodies in the patient's sample other than anti-A or anti-B.

4.2.3. To prevent misinterpretation of results due to haemolysis where serum is used, it is recommended that the diluent used for resuspension of reverse grouping cells contains EDTA. See 8.3.2.

4.3. RhD grouping

4.3.1. Each sample must be tested in duplicate with IgM monoclonal anti-D blood grouping reagents which should not detect D^{VI}.

4.3.2. The antiglobulin test *should not* be used for RhD grouping.

4.4. Controls

4.4.1. Positive and negative controls must be included with each batch of tests, as follows:

Reagent	Positive control cells	Negative control cells
Anti-A	A	B
Anti-B	B	A
Anti-D	RhD positive	RhD negative

4.4.2. A reagent control should be used where recommended by the manufacturer.

4.5. Interpretation of results

4.5.1. *Manual reading.* Documentation errors may occur during the manual reading and interpretation of ABO and RhD groups. The risk of error can be minimized by separating the procedure into distinct tasks and wherever possible using different members of staff to perform each task. Suggested options for achieving this are: (i) separating the documentation of reaction patterns from the final interpretation; (ii) separating the interpretation and documentation of the cell and reverse groups.

4.5.2. *Automated reading.* Automated readers are frequently used to interpret individual reactions and reaction patterns, when using microplate or microcolumn techniques. The system must be validated against manual systems prior to routine use.

In the absence of a *fully* automated system (e.g. where there is no integrated barcode reader), procedures including double checking of samples and plates or cards should be in place to prevent misidentification.

A visual inspection of results is still necessary when using automated readers which are unable to interpret mixed field reactions.

4.6. Verification of results

4.6.1. The ABO and RhD group must, wherever possible, be verified against previous results for the patient.

4.6.2. Any discrepancies must be resolved prior to transfusion of red cells or red cell contaminated components.

4.7. Grouping anomalies. The following are all examples of blood group anomalies.

4.7.1. *Cold auto-antibodies.* See section 4.2.2.

4.7.2 *Acquired B.* Some anti-B reagents may react strongly with the acquired B antigen. This usually leads to a discrepancy between call and reverse groups. If, however, the patient's own anti-B is only weakly detectable, an incorrect interpretation may result. Particular care must be taken if an anti-globulin test is not performed as part of the cross-match.

4.7.3. *Unexpected mixed field reactions.* Any samples showing mixed field reactions must be repeated and/or investigated prior to group authorization or issue of red cells.

4.7.4. *Partial or weak D.* See section 4.9.

4.7.5. *Intrauterine transfusions.* For a period of several months post delivery, neonates who have received intrauterine transfusion may appear to be the same ABO and RhD group as that of the transfused red cells due to bone marrow suppression.

4.8. Repeating ABO and RhD grouping

4.8.1. ABO and RhD groups must be repeated when a discrepancy is found.

4.8.2. Repeats should be performed using washed cells. To prevent the perpetuation of mistakes, the cells used should be taken from the original sample, rather than from a suspension made previously. An auto control should be included.

4.8.3. Repeatably anomalous results should be referred to a senior person in the laboratory. It may also be necessary to obtain a fresh sample and refer to a reference laboratory.

4.8.4. If it is not possible to obtain a reliable reverse grouping result due to the age of the patient or to insufficient sample, and there is no historical group against which to validate, the cell group must be repeated.

4.8.5. Where verification checks against historical results reveal a discrepancy, a further sample must be obtained and tested immediately.

4.9. Partial and weak D

4.9.1. It is important to note that monoclonal anti-D reagents vary widely in their ability to detect both partial and weak D. It may be helpful to use reagents which give similar reactions. Where there is a discrepancy in typing, the patient should be treated as RhD negative until the D status is resolved. The sample may need to be sent to a reference laboratory for investigation.

4.9.2. Patients with a known partial D status should be regarded as RhD negative.

4.9.3. Patients with a weak D status may be regarded as RhD positive.

4.9.4. Patients of category D^{VI} status are those most likely to make anti-D, reagents used for RhD grouping of patients must not detect category D^{VI}.

4.10. Infants. 4.10.1 It is important to distinguish cord samples from maternal samples to prevent mis-typing.

4.11. ABO/RhD grouping in urgent situations

4.11.1. When blood or blood products are required urgently, there may be insufficient time for routine ABO/RhD grouping prior to selection of blood products.

4.11.2. Emergency groups performed in these circumstances must include a test against anti-A, anti-B and anti-D, with appropriate controls or a reverse group.

4.11.3. The result must be documented, and confirmed as soon as possible by routine methods if these differ from emergency procedures.

5. ANTIBODY SCREENING

5.1. Introduction

5.1.1. Antibody screening undertaken in advance of the requirement to provide blood for transfusion alerts the clinician to possible delay in the supply of compatible blood if the antibody screen is positive. It also provides the laboratory with time to identify irregular antibodies and select suitable units.

5.1.2. Antibody screening may be more reliable and sensitive than crossmatching against donor red cells and it is therefore recommended that antibody screening should be performed in all pretransfusion testing (see also 5.3.1.).

5.2. Choice of techniques

5.2.1. *Indirect antiglobulin test (IAT).* The IAT using red cells suspended in low-ionic-strength saline (LISS) is considered to be the most suitable for the detection of clinically significant antibodies, because of its speed, sensitivity and specificity. Liquid-phase tube and microplate, solid phase microplate and microcolumn ('column agglutination') antiglobulin methods have all been shown to be reliable. The use of normal-ionic-strength techniques (NISS) requires a minimum incubation time of 45 m and is therefore not recommended, but may be useful when particular problems with LISS are encountered, for example when LISS-dependent auto-antibodies are present or there are other non-specific reactions. See 6.8.6.

5.2.2. *Antibody screening using the IAT alone.* Since IAT methods can detect almost all clinically significant antibodies, it is acceptable to use an IAT for pretransfusion antibody screening without any additional screening technique. The use of an unsupported IAT should only be implemented if: (i) the laboratory has implemented a documented programme for the assessment of worker proficiency in the IAT method; (ii) the laboratory has implemented a documented

programme of replicate testing to assure the efficacy of cell washers; (iii) the IAT method has been properly validated against the documented requirements of the laboratory; (iv) the laboratory has performed consistently well in NEQAS exercises using different workers and the IAT method in use.

A fully automated screening IAT method, including positive sample identification at the sampling and reading stages, provides a valuable additional security check if a single antibody screening method is used. It should be recognized that nonautomated methods are more liable to human error.

5.2.3. *Additional techniques.* Additional techniques, such as two-stage enzyme and Polybrene methods may be used. However, it must be realized that these methods are unable to detect with an adequate level of sensitivity as wide a range of specificities as the IAT is capable of, and proficiency in the performance in the IAT is therefore of over-riding importance.

References for these methods will be found in Mollison *et al.*, (1993) and Scott *et al.* (1994).

5.3. Reagent red cells for use in antibody screening

5.3.1. Antibody screening provides the laboratory with the most reliable and sensitive method of detecting an irregular red cell antibody. Crossmatch methods using red cells from donor units are often less reliable because the expression of blood group antigens varies according to genotype: for example, the homozygous genotype Jk^aJk^a often results in a higher expression of the Jk^a antigen than the heterozygous genotype Jk^aJk^b. In addition, red cells for antibody screening should be preserved in a medium shown to minimize loss of blood group antigens during the recommended storage period. For these reasons, an antiglobulin crossmatch using donor cells is not the most effective way of detecting a serological incompatibility between patient and donor.

5.3.2. The specification for red cells suitable for use in antibody screening are summarized below.

5.3.2.1. The following antigens should be expressed: C, c, D, E, e, K, k, Fy^a, Fy^b, Jk^a, Jk^b, S, s, M, N, P_1, Le^a, Le^b.

5.3.2.2. Reagent red cells should not be pooled.

5.3.2.3. At least one of the reagent red cell samples should express the probable haplotype R_2.

5.3.2.4. Apparent homozygous expression of the following, in the stated order of priority, is desirable: D, c, Fy^a, Jk^a, Jk^b, S, s, Fy^b.

5.3.3. It is essential that if an antiglobulin crossmatch against donor red cells has been omitted the sensitivity of the antibody screening system in use is at least equivalent to that obtained with a LISS spin IAT test using reagent red cells having homozygous expression of all the antigens listed in 5.3.2.4.

5.4. Autologous controls. An autologous control or direct antiglobulin test (DAT) need not form a part of antibody screening.

6. ANTIBODY IDENTIFICATION

6.1. When an irregular antibody is detected in the screening procedure, its specificity should be determined and its clinical significance assessed.

6.2. If the patient is known to have a red cell antibody, the serum/plasma should be checked on each occasion of testing to exclude the development of further alloantibodies.

6.3. A blood sample should be referred to a red cell reference laboratory if there is any doubt concerning the identity of the antibody/ies present or lack of exclusion of clinically significant antibodies.

6.4. Laboratories which are not registered for antibody identification in NEQAS should refer all sera which have given positive results in the antibody screen to a laboratory which is registered for antibody identification.

6.5. It is important to recognize the limitations of the panel in use. A single panel may be unable to identify some common combinations of antibodies, and the use of a second panel is strongly recommended for laboratories which do not refer samples to a reference laboratory so that additional antibodies of clinical significance can be excluded.

6.6. Principles of antibody identification

6.6.1. The patient's serum should be tested by an appropriate technique against an identification panel of reagent red cells. As a starting point, the technique by which the antibody was detected during screening should be used. Inclusion of the patient's own red cells may be helpful, for example in the recognition of an antibody directed against a high-frequency antigen.

6.6.2. The specificity of the antibody should only be assigned when it is reactive with at least two examples of reagent red cells carrying the antigen and nonreactive with at least two examples of reagent red cells lacking the antigen.

6.6.3. When one antibody specificity has been identified it is essential that the presence of additional clinically significant antibodies have not been missed. Multiple antibodies can only be confirmed by choosing cells antigen negative for the recognized specificity but positive for other antigens to which clinically significant antibodies may arise.

6.6.4. The use of additional techniques, for example enzyme and low-temperature saline techniques, may be helpful in antibody identification, particularly when an antibody weakly reactive by antiglobulin,

or a mixture of antibodies, is present. The use of monospecific antiglobulin reagents in place of polyspecific reagent is beneficial when determining the presence of IgG antibodies in serum samples containing complement binding antibodies.

6.6.5. Although most antibodies detectable only by an enzyme technique are unlikely to be of clinical significance, specific antibodies should not be ignored unless procedural errors in the antiglobulin test have been ruled out. This can be best achieved by retesting the serum/plasma against homozygous cells by an antiglobulin technique.

6.6.6. The patient's red cells should be phenotyped using antiserum of appropriate specificity. The incorporation of a reagent control or AB serum control used by the same technique as the phenotyping serum is particularly important. A positive DAT will invalidate test results.

6.7. Reagent red cells for use in antibody identification. Specifications for suitable red cells for use in antibody identification are summarized below.

6.7.1. The panel should permit confident identification of those clinically significant alloantibodies which are most frequently encountered, for example, anti-D, anti-E, anti-c, anti-K and anti-Fy^a.

6.7.2. A distinct pattern of reactivity should be apparent for each of the commonly encountered alloantibodies.

6.7.3. The antigenic profile of the reagent red cells should, as far as possible, permit assignment of specificity in test sera containing more than one commonly encountered alloantibody, for example anti-D + K.

6.7.4. *Minimum* characteristics are: (i) one individual should be R_1R_1 and one $R_1{}^wR_1$. Between them, these two individuals should express the antigens: K, k, Fy^a, Fy^b, Jk^a, Jk^b, S, s; (ii) one individual should be R_2R_2 and one r′r; (iii) A minimum of four individuals should lack the Rh antigens C and D. One of these individuals should be K+ and one should be E+. Between then these individuals should exhibit apparent homozygous expression of: c, k, Fy^a, Fy^b, Jk^a, Jk^b, S, s.

6.8. Autoantibodies

6.8.1. Many autoantibodies cause no clinical problems. In patients with autoimmune haemolytic anaemia (AIHA), autoantibodies directed against red cell antigens are responsible for shortening red cell survival which may lead to severe anaemia.

6.8.2. Serological investigations in AIHA should focus on the determination of the correct ABO and RhD group of the patient and determination of the presence of alloantibody. Autoantibody may 'mask' the presence of underlying alloantibody. *It may be necessary to refer cases of AIHA to a red cell reference laboratory in view of the complexity of the investigations required.*

6.8.3. Selection of blood for transfusion may be influenced by the presence of an autoantibody of 'simple' specificity, but extensive investigations to determine complex specificities of autoantibodies are rarely of value.

6.8.4. Cold-type AIHA or cold haemagglutinin disease.

6.8.4.1. The red cells from the patient should be washed at 37°C for performing the DAT.

6.8.4.1. The red cells from the patient will usually have a strongly positive DAT due to coating with complement (C3d) components.

6.8.4.2. Reagent controls are particularly important when phenotyping the patient's red cells or performing the DAT because of the possibility of autoagglutination.

6.8.4.3. It is important to exclude the presence of alloantibodies using cells and serum separately prewarmed to 37°C; the use of anti-IgG in place of polyspecific antiglobulin reagent may also be helpful when serum not plasma is used.

6.8.4.4. It should be noted that some antibodies of apparently clear-cut specificity, for example anti-M, may be *auto* in nature.

6.8.5. Warm-type AIHA.

6.8.5.1. The red cells from the patient will usually have a positive DAT due to coating with IgG and sometimes complement components.

6.8.5.2. Phenotyping of the patient's red cells can only be performed using IgM or chemically modified IgG (complete) saline reactive antisera. Reagent controls are essential. IgG antibody may be removed from the red cells by, for example, treatment with chloroquine diphosphate; however, results should be interpreted with caution as antigens can be removed or destroyed (Edwards *et al.*, 1982).

6.8.5.3. *Autoabsorption* using the patient's red cells may be necessary to permit the recognition of underlying alloantibody. Removal of autoantibody from the patient's red cells and enzyme treatment of the cells improves the efficiency of autoabsorption and may be performed in a single stage using the ZZAP method (Branch & Petz, 1982).

6.8.5.4. In some circumstances, autoabsorption may be difficult, or undesirable (e.g. following a recent transfusion). Absorption with red cells which are Rh identical, K− and if possible Fy(a−−) and Jk(a−−) should allow the exclusion of most antibodies of clinical significance. If a more complete phenotype of the patient's red cells is known, as close a match as possible for the absorbing red cells should be used.

6.8.6. *'LISS-dependent antibodies'.* Some sera/plasma

will be found to contain an antibody, usually of no particular specificity, which reacts with red cells suspended in LISS but not in NISS. These antibodies may be directly agglutinating and are usually complement binding. If plasma is used subsequent serological work may be performed using NISS; if serum is used, anti-IgG should replace the polyspecific antiglobulin reagent.

7. CROSSMATCHING

7.1. *Introduction.* The crossmatch is defined in this document as a procedure to exclude incompatibility between donor and recipient. This may include serological tests or electronic crossmatching. See 7.6.

7.2. *Selection of blood*

7.2.1. Red cell components of the same ABO and RhD group as the patient must be selected whenever possible. If ABO identical blood is not available, group O blood may be used provided it is plasma depleted or does not contain high-titre haemagglutinins. Group AB blood should be used for AB patients but if it is not available group A or B red cells may be used.

When supplies of RhD-negative blood are limited, RhD-positive blood may be selected for RhD-negative recipients. It is important that RhD-positive cellular components should not be issued to RhD-negative premenopausal females.

7.2.2. *Patients with clinically significant red cell antibodies.* Blood should be selected which has been tested and found negative for the relevant antigen. If the antibodies are not clinically significant, it is not necessary to select antigen negative blood (see section 1.3).

7.2.3. *Patients with autoimmune haemolytic anaemia.* Except in emergency situations, patients should be investigated for the presence of alloantibodies as in section 6.8. It is unacceptable simply to crossmatch and issue blood as compatible as the patient's own cells and serum.

7.2.4. *Massive blood transfusion.* Where the volume of blood transfused in any 24-h period is equivalent to the patient's own blood volume, ABO group identical blood can be issued without further serological testing. The laboratory staff must assure themselves of the validity of the ABO and RhD group of the donor blood. If ABO nonidentical blood has to be transfused, blood of the same group as the patient should be used as soon as possible. There is no need to persist with the ABO group originally transfused.

7.2.5. *Fetal/neonatal transfusions.* (i) *Fetal transfusion.* Blood should be crossmatched against the maternal serum/plasma; this should include an indirect antiglobulin test if the maternal serum/plasma contains clinically significant red cell antibodies. The blood should be less than 5 days old, group O, of high haematocrit (0·55–0·75), CMV negative and irradiated. (ii) *Neonatal exchange transfusion.* If group O blood is selected irrespective of the baby's ABO group, the laboratory should seek assurance that high-titre anti-AB in the donation has been excluded. The blood should be used within 5 days of collection. (iii) *Neonatal top up transfusion.* In the absence of atypical maternal antibodies blood may be given without prior crossmatching.

See Guidelines for administration of blood products; transfusion of infants and neonates (BCSH; 1994).

7.2.6. *Sickle cell disease.* The incidence of alloimmunization in multiply transfused sickle cell anaemia patients varies from about 10% in children to 50% is some adult populations with a general range of 20–30% (Ness, 1994). It is desirable to phenotype sickle cell patients as fully as possible prior to transfusion and to match for K, C and E antigens before the onset of alloimmunization. Other extended antigen matching may be required.

7.2.7. *Chronically transfused patients.* In contrast to patients with sickle cell disease, other groups requiring chronic transfusion are not at excessive risk of alloimmunization and phenotyping and antigen matching are not necessary.

7.2.8. *Recipients of allogeneic haemopoietic stem cell grafts.* Recipients of allogeneic transplants present unusual grouping and crossmatching problems. The transplant may introduce a new ABO antigen (major mismatch) or a new ABO antibody (minor mismatch) or both. All cellular products should be irradiated to prevent graft versus host disease. See Guidelines on gamma irradiation of blood components for the prevention of graft-versus, host, disease (BCSH, 1996). (i) *Major ABO mismatch.* Red cells should be of the patient's own ABO group until recipient ABO group is no longer detectable and the DAT is negative. (ii) *Minor ABO mismatch.* Red cells should be of donor ABO group and plasma depleted until the original recipient red cells are no longer detectable. (iii) *Combined ABO mismatch.* Red cells should be group O until recipient ABO group is no longer detectable. (iv) *RhD-positive recipient with RhD-negative donation or graft.* RhD negative components should be transfused.

7.3. Procedure

7.3.1. It is preferable for one person to carry out the crossmatching procedure from beginning to end. Where this is not possible there should be written auditable procedures to establish staff accountability.

7.4. Immediate spin crossmatch

7.4.1. The immediate spin crossmatch *must not* be used alone: (i) if the patient's serum/plasma contains or has been known to contain clinically significant antibodies; (ii) if the antibody screening test does not conform to the recommendations in section 5; (iii) if ABO grouping reveals macroscopically undetectable anti-A or anti-B, except in group AB patients; (iv) except in emergency situations. See 7.7.

7.4.2. A short incubation time of 2–5 min before centrifugation is recommended to enhance the detection of weak ABO antibodies. EDTA saline is recommended to overcome the potential problem of prozone if serum is used. (Judd *et al.*, 1988).

7.5. IAT crossmatch

7.5.1. A crossmatch which includes an indirect antiglobulin test *must* be used if: (i) the patient's plasma/serum contains or has been known to contain clinically significant red cell alloantibodies; (ii) the antibody screen does not conform to the recommendations in section 5; (iii) ABO grouping reveals macroscopically undetectable anti-A or anti-B, except in group AB patients; (iv) the patient has had an ABO incompatible solid organ transplant and is being transfused within three months of the transplant. This is necessary to detect IgG anti-A or anti-B produced by passenger lymphocytes in the transplanted organ.

7.6. Computer issue without a serological crossmatch

7.6.1. A cautionary approach must be taken before considering the implementation of this innovation. See Appendix 2 for minimum recommendations.

7.7. Emergency situations

7.7.1. A correctly labelled blood sample must be obtained from the patient. If a correctly labelled sample is not immediately available and blood is required because of a life-threatening situation, group O blood must be issued. If the patient is a premenopausal female, group O RhD-negative blood must be given.

7.2.2. The sample should be ABO and RhD grouped by rapid techniques.

7.7.3. Blood of appropriate ABO and RhD group may be issued after completion of a reverse group, repeat cell group or immediate spin crossmatch.

7.7.4. The laboratory must ensure themselves of the validity of the ABO and RhD group of the donor blood. This will require written verification from the supplying Blood Transfusion Centre or confirmatory testing within the hospital's laboratory.

7.7.5. An antibody screen should be performed as soon as possible. If this is negative it is not necessary to carry out a retrospective IAT crossmatch.

7.8. The compatibility report

7.8.1. A compatibility report must be issued before or with the first unit of blood. Information should include the location of the laboratory, the patient's surname, first name, hospital no., date of birth, ward and blood group of the patient and the donation number and blood group.

7.8.2. If standard pretransfusion testing has not been carried out this should be stated in the report. The report may be used as a record in the patient's notes that pretransfusion testing has not been carried out in full.

7.9. The compatibility label. 7.9.1 There must be a compatibility label which should be securely attached to the blood bag. Information should include the patient's surname, first name, hospital number, donation number and group, and the date the blood is required/crossmatched.

7.10. Visual inspection of the red cell unit. 7.10.1 Before the unit is placed in the blood issue fridge it should be inspected for: (i) integrity of the pack by checking for leaks at the ports and seams; (ii) evidence of haemolysis in the plasma or at the interface between the plasma and red cells; (iii) evidence of discoloration of the red cells; (iv) presence of large clots in the pack.

If there is any evidence of the above the unit should not be used and should be returned to the issuing Blood Transfusion Centre.

7.11. Removal of blood from the issue refrigerator should be in accordance with a written procedure.

8. TECHNIQUES

8.1. Introduction

8.1.1. As new techniques are continually evolving it is not possible to provide a comprehensive list of recommended methods. Users of new technologies should follow the manufacturer's instructions and should refer to the current BCSH Guidelines for evaluation, validation and implementation of new techniques for blood grouping, antibody screening and crossmatching (BCSH, 1995).

8.1.2. Guidance for microplate methods may be found in the BCSH Guidelines for microplate techniques in liquid phase blood grouping and antibody screening (BCSH, 1991c).

8.1.3. Particular emphasis must be placed on the interpretation of weak reactions when establishing or reviewing a procedure and during training, so that inconsistency is minimized. This must then be consolidated by ongoing quality assurance for staff, equipment and reagents (see Section 2).

8.2. Antiglobulin techniques

8.2.1. Method – see Appendix 1.

8.2.2. Monospecific anti-IgG may be used in place of

polyspecific AHG for the LISS IAT because of the higher sensitivity of the LISS IAT methods and because of the increased, undesirable susceptibility of these methods to interference from low thermal optimum and LISS-dependent antibodies that are complement binding. Polyspecific antiglobulin reagent confers no advantage over monospecific anti-IgG if plasma is used for antibody screening. However, it is important that screening cells having homozygous expression of Jk^a can be guaranteed by the supplier before a decision is taken to use anti-IgG in place of polyspecific antiglobulin reagent.

8.2.3. IgG-sensitized control red cells for the techniques above should be added to all negative tests to confirm the efficacy of the washing stage. In order to be fully effective, the level of IgG sensitization should be limited to that which gives a macroscopically negative result after the addition of 0.1% v/v serum in saline to the antiglobulin reagent.

8.3. *Solutions*

8.3.1. Phosphate-buffered saline (PBS), normal saline and LISS solutions. Reference should be made to the guidelines for the Blood Transfusion Services in the UK (Department of Health, 1994).

8.3.2. *EDTA for diluents.* Stock solution: prepare a 0·1 M solution of EDTA (di-potassium salt) in distilled water. Adjust the pH to 7·0 using 5 M NaoH. Working solution: mix one volume of stock solution with nine volumes of saline or LISS. Check the pH and adjust to 7·0 if necessary.

REFERENCES

BCSH (1991a) Compatibility testing in hospital blood banks. In: *Standard Haematology Practice* (ed. Roberts, B.), 150–163. Blackwell Scientific Publications, Oxford.

BCSH (1991b) Hospital blood bank documentation and procedures. In: *Standard Haematology Practice* (ed. Roberts, B.), 128–138. Blackwell Scientific Publications, Oxford.

BCSH (1991c) Microplate techniques in liquid phase blood grouping and antibody screening. In: *Standard Haematology Practice* (ed. Roberts, B.), 164–188. Blackwell Scientific Publications, Oxford.

BCSH (1994) Guidelines for administration of blood products: transfusion of infants and neonates. *Transfusion Medicine*, **4**, 63–69.

BCSH (1995) Guidelines for evaluation, validation and implementation of new techniques for blood grouping, antibody screening and crossmatching. *Transfusion Medicine*, **5**, 145–150.

BCSH (1996) Guidelines on gamma irradiation of blood components for the prevention of transfusion-associated graft-versus host disease. *Transfusion Medicine*, **6**, 261–271.

Branch, D.R. & Petz, L.D. (1982) A new reagent (ZZAP) having multiple applications in immunohematology. *American Journal of Clinical Pathology*, **78**, 161–167.

Department of Health (1994) *Guidelines for the Blood Transfusion Services in the United Kingdom*. HMSO, London.

Edwards, J.M., Moulds, J.J. & Judd, W.J. (1982) Chloroquine dissociation of antigen–antibody complexes. A new technique for typing red blood cells with a positive direct antiglobulin test. *Transfusion*, **22**, 59–61.

Judd, W.J., Steiner, E.A., O'Donnell D.B. & Oberman, H.A. (1988) Discrepancies in reverse ABO typing due to prozone. How safe is the immediate-spin crossmatch? *Transfusion* **28**, 334–338.

Mollison, P.L., Engelfriet, C.O. & Contreras, M. (1993) *Blood Transfusion in Clinical Medicine*, 9th end. Blackwell Scientific Publications, Oxford.

Ness, P.M. (1994) To match or not to match: the question for chronically transfused patients with sickle cell anaemia. *Transfusion*, **34**, 558–560.

Phillips, P.K., Voak, D., Whitton, C.M., Downie, D.M., Bebbington, C. & Campbell, G. (1993) BCSH-NIBSC anti-D reference reagent for antiglobulin tests: the in-house assessment of red cell washing centrifuges and of operator variability in the detection of weak, macroscopic agglutination. *Transfusion Medicine*, **3**, 143–148.

Sazama, K. (1990) Reports of 355 transfusion-associated deaths: 1976 through 1985. *Transfusion*, **30**, 583–590.

Scott, M., Voak, D., Phillips, P.K., Hoppe, P.A. & Kochman, S.A. (1994) Review of the problems involved in using enzymes in blood group serology – provision of freeze dried ICSH/ISBT protease enzyme and anti-D reference standards. *Vox Sanguinis*, **67**, 89–98.

Voak, D., Downie, D.M., Moore, B.P.L., Ford, D.S., Engelfriet, C.P. & Case, J. (1988) Replicate tests for the detection and correction of errors in anti-human globuline (AHG) tests: optimum conditions and quality control. *Haematologia*, **21**, 3–16.

Voak, D. (1992) Validtion of new technology for antibody detection by antiglobulin tests. *Transfusion Medicine*, **2**, 177–179.

APPENDIX 1

Methods for the antiglobulin technique

	Technique used	
	LISS tube	NISS tube
Serum: cell suspension ratio*	2:2	4:1
Red cell concentration	1·5–2%	2–3%
Incubation time (min)	15–60	45–90
No. of saline washes	4	4
Volume of antiglobulin reagent	70–100 μL	70–100 μL
Centrifugation time/speed	In-house validation or as recommended by the manufacturer	
Reading method	Tip and roll	Tip and roll

* Total volume of serum should not exceed 200 μL. It may be helpful to use a reading aid such as a hand lens, microscope or concave mirror to assist in the interpretation of test results obtained in liquid phase tube and microplate methods.

APPENDIX 2

Minimum recommendations for computer issue without a serological crossmatch

Computer systems *should not* be considered for detecting an ABO mismatch unless the Consultant responsible for the Blood Transfusion laboratory has ensured that the following minimum recommendations are met.

(i) An automated system for ABO and RhD grouping and antibody screening including positive sample identification and electronic data transfer of results is in place.

(ii) The antibody screening procedure conforms to the recommendations in section 5.

(iii) The patient's plasma/serum does not or has not been known to contain clinically significant red cell alloantibodies.

(iv) Computer software is validated to ensure that the criteria in (v) are met.

(v) The release of ABO-incompatible blood must be prevented by conformation of the system to the following requirements.

- The issue of blood is not allowed if there is only one ABO and RhD group on file.
- The issue of blood is not allowed if the current blood group does not match the historical record on file.
- The historical results of ABO, RhD, and antibody screening must not be displayed when manual entry of current results is made.
- The system must not permit the reservation, and release of red cell units that are ABO incompatible with the ABO group of the patient.

(vi) The laboratory must assure the validity of the ABO and RhD group of the donor blood. This will require written verification from the supplying Blood Transfusion Centre or confirmatory testing within the hospital laboratory.